Mucosal Iodine Staining Improves Endoscopic Visualization of Squamous Dysplasia and Squamous Cell Carcinoma of the Esophagus in Linxian, China

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BACKGROUND. In previous studies in the high risk population of Linxian, China, the majority of foci of high grade (moderate and severe) squamous dysplasia (HGD) and invasive squamous carcinoma (CA) of the esophagus were associated with endoscopically visible lesions that could be targeted for biopsy, but some foci of HGD were missed by routine endoscopic examination. This study examined whether spraying the mucosa with Lugol's iodine solution, which stains normal epithelium brown but leaves dysplasia and carcinoma unstained, could improve endoscopic detection and delineation of these lesions.

METHODS. Two hundred twenty-five Linxian adults with balloon cytologic evidence of dysplasia or carcinoma underwent endoscopy. All visible lesions were described and photographed before and after staining with 1.2% Lugol's iodine solution. Biopsies were taken from all lesions visible before staining, from all unstained lesions (USLs) after applying the stain, and from representative control areas of stained mucosa.

RESULTS. Two hundred fifty-three USLs and 255 control sites were biopsied. No complications occurred. Ninety-four biopsy sites contained HGD and 20 contained CA. Before staining, the sensitivity of visible lesions for identifying HGD or CA was 62%, and the specificity was 79%. After staining, the sensitivity of USLs for identifying HGD or CA was 96%, and the specificity was 63%. Eighty-eight percent of the HGD and CA lesions were larger or more clearly defined after staining. The diagnostic lesions in 17 of 31 patients with moderate dysplasia (55%), 8 of 35 patients with severe dysplasia (23%), and none of the 19 patients with invasive carcinoma (0%) were identified only after staining.

CONCLUSIONS. Mucosal iodine staining improved endoscopic detection and delineation of HGD and CA in these patients. This simple technique is highly sensitive for identifying these precursor and invasive squamous lesions, and it should be used whenever optimal visualization of squamous mucosal abnormalities is required. ***Cancer* 1998;83:220–31.** © *1998 American Cancer Society.*

KEYWORDS: endoscopy, iodine staining, early detection, esophageal neoplasms, squamous cell carcinoma, precursor lesions, China.

Esophageal carcinoma is a common malignancy with a very poor prognosis.[1] It is the fourth most common cause of cancer death in China, the fourth most common cause of cancer death in African-American men, and the eighth most common cause of cancer death in American men of all races.[2,3] Between 1983–1990, the 5-year relative survival rate for esophageal carcinoma in the U. S. was 9.2%, among the lowest for all cancers.[3] The main reason for this poor survival is that the majority of esophageal carcinomas are asymptomatic and go undetected until they have spread beyond the esophageal wall and are unresectable. In this setting, there is a clear need for improved strategies for detection and curative treatment of precursor

lesions and early invasive esophageal tumors. The current study evaluates a mucosal staining technique for visualizing precursor and early invasive lesions of esophageal squamous cell carcinoma, which is the predominant histologic type of esophageal carcinoma in all high risk areas throughout the world and accounts for 50–60% of esophageal carcinoma in the U. S.[4]

During the past 15 years, Chinese and American researchers have collaborated in the conduct of two nutrition intervention clinical trials in Linxian, a county in Henan Province in northcentral China that has very high rates of esophageal squamous cell carcinoma.[5] As part of these trials, we have performed several studies relevant to the development of practical early detection strategies for these tumors. In one of these studies, we found that the majority (73%) of the biopsies of high grade (moderate and severe) squamous dysplasia and all biopsies of invasive squamous cell carcinoma came from endoscopically visible mucosal lesions, but a significant minority (27%) of the sites containing high grade dysplasia could not be identified visually by routine endoscopic examination.[6]

For several years, Japanese and European authors have reported that staining the esophageal mucosa with Lugol's iodine solution can make the presence and extent of squamous dysplastic and cancerous foci more clear,[7–26] but this technique has not often been used by Chinese or American endoscopists. The purpose of this study was to evaluate whether mucosal iodine staining could improve the detection and delineation of high grade squamous dysplasia and squamous cell carcinoma of the esophagus in Linxian.

MATERIALS AND METHODS

The general design of this study was to endoscope 225 Linxian adults with cytologic evidence of dysplasia or carcinoma; to describe and photograph all visible mucosal lesions before and after staining with 1.2% Lugol's iodine solution; to biopsy all lesions visible before staining, all unstained areas after staining, and at least one control area of stained mucosa in each patient; to estimate the sensitivity of mucosal lesions visible before staining and the sensitivity of unstained areas after staining for detecting foci of high grade squamous dysplasia and invasive squamous cell carcinoma; and to estimate the proportion of patients with high grade dysplasia or carcinoma who were detected only after staining. This study was approved by the Institutional Review Boards of the collaborating institutions, the Cancer Institute of the Chinese Academy of Medical Sciences, Georgetown University, and the U. S. National Cancer Institute.

Patient Population

Participants were recruited in September, 1994 in Donggang commune, Linxian County and in Dongshui village, Anyang County. All individuals in Donggang commune and Dongshui village who were ages 40–69 years and had no contraindication to balloon cytology or endoscopy were invited to participate. Two thousand and forty-three subjects, approximately 80% of those eligible, agreed to take part and provided their informed consent. These subjects were given a simple baseline interview, with questions regarding symptoms and allergies, and then referred for cytologic examination.

Cytologic Examinations

All subjects were screened by esophageal balloon cytology, using a double lumen balloon and routine collection and processing methods.[27] Four direct smears were made per subject. All slides were read by experienced Chinese cytotechnologists, using Chinese cytologic categories and criteria (which include more cases as precancerous neoplasia than do Western cytologic criteria).[28,29] There were only 9 cases (0.4%) deemed unsatisfactory for diagnosis. There were 164 cases of high grade dysplasia (the Chinese categories of Dysplasia 2 and Near Cancer) (8.0%), and 72 cases of carcinoma (3.5%) among the 2043 subjects who were screened.

Endoscopic Examinations

Of 236 eligible patients (those with cytologic diagnoses of high grade dysplasia or carcinoma in the September balloon screening), 225 (95%) underwent endoscopy between October 11 and October 23 1994. The patients were given 5 mL of a 1% dicaine slurry to drink for local anesthesia 2–5 minutes before endoscopy, but were not otherwise sedated. Endoscopy was performed by two of the authors (D.E.F. and G.Q.W.), using an Olympus GIF-130 or a Pentax EG-2900 videoendoscope (Olympus Corporation, Tokyo, Japan; Pentax Corporation, Orangeburg, NY), without knowledge of the cytology results. Digital photographs were stored in an Olympus ImageManager or a Pentax IMS-3000 computer, transported on computer disks, and printed by a Sony Mavigraph thermal printer (Sony Corporation, Tokyo, Japan).

The entire esophagus and stomach were examined, and all visually abnormal areas were described and photographed. The endoscopic appearances before staining were categorized as follows[6]: Normal: the mucosa was smooth or mildly wrinkled, with no abnormalities. Irregular: the mucosa was focally or diffusely irregular, with prominent wrinkling. Small White Patch: there was a focal raised or flat white

patch, with smooth distinct borders, usually < 1 cm in greatest dimension. Focal Red Area: there was a focal flat red area not caused by mucosal contact. Erosion: there was a focal defect in the mucosa; the erosions were subcategorized as linear, punched-out, or broad-based, depending on their shape and size. Plaque: the mucosa was focally thickened and raised, with irregular indistinct borders and occasional shallow surface erosions; plaques usually were >1 cm in greatest dimension. Nodule or Obstructing Tumor: there was a macroscopic tumor protruding into the lumen.

The upper and lower borders of visible lesions were recorded as the distance from the incisor teeth, and the circumferential extent of lesions was recorded in quarters (1–25%, 26–50%, 51–75%, and 76–100%).

After the initial inspection, 20–30 mL of 1.2% glycerin free Lugol's iodine solution (12 g iodine + 24 g potassium iodide in 1000 mL water) was sprayed from the gastroesophageal junction to the upper esophageal sphincter using a plastic spray catheter (washing tube PW-5L; Olympus Corporation) passed through the biopsy channel. After iodine spraying, the esophagus was examined again, and mucosal areas were categorized as unstained, normally stained, or overstained. All unstained and overstained areas were described and photographed. More detailed descriptions of the staining pattern of unstained areas (light yellow, dark yellow; mosaic staining; etc.) occasionally were noted but were not recorded systematically. The upper and lower borders and circumferential extent of unstained and overstained areas were recorded as described earlier. The relative size of lesions and the relative clarity of lesion borders before and after staining also were recorded. One or more 2.8-mm biopsies were taken from all lesions that were visible before staining, from all but very small unstained areas, from some overstained areas, and from at least one normally stained site, either near an unstained lesion (a control biopsy) or from the midesophagus (a standard biopsy). Gastric biopsies were taken from all lesions that were observed before staining (staining did not reveal additional gastric lesions). At the end of the procedure, the stomach was suctioned to remove excess iodine and air.

Biopsy Slide Reading

The biopsies were oriented mucosal side up on filter paper supports,[30] fixed in 10% neutral-buffered formalin, embedded in paraffin, cut in 5-μm sections, and stained with hematoxylin and eosin. The biopsies were read independently by three of the authors (S.M.D., N.L., and K.J.L.) and discrepancies were resolved by simultaneous review at a three-headed microscope, without knowledge of the cytology results or the endoscopic findings. The esophageal biopsies were categorized as follows[31,32]: Normal: there was a well oriented squamous epithelium, without evidence of esophagitis, squamous dysplasia, or squamous carcinoma. Esophagitis: one or more of the following three criteria were present: 1) elongation of lamina propria papillae into the upper third of the epithelium together with basal cell hyperplasia, defined as a basal zone thickness of > 15% of total epithelial thickness, 2) epithelial infiltration by neutrophils or eosinophils, or 3) a dense, nonfollicular mononuclear infiltrate or an easily recognized infiltrate of neutrophils in the lamina propria. Squamous Dysplasia: nuclear atypia (enlargement, pleomorphism and hyperchromasia), loss of normal cellular polarity, and abnormal tissue maturation were present in the lower third (mild), in the lower two-thirds (moderate), or in all thirds (severe) of the epithelium. Squamous Carcinoma: malignant squamous cells were present that had invaded through the basement membrane.

The gastric biopsies were categorized as normal, gastritis, low grade dysplasia, high grade dysplasia, or invasive adenocarcinoma, as previously described.[32]

Analysis

The results were analyzed separately by biopsy site and by patient. In the biopsy site analysis, the endoscopic appearances before and after staining were compared with the worst biopsy diagnosis from each site. For the comparison before staining, all endoscopic categories except Normal were considered visible lesions. For the comparison after staining, normally stained sites (N = 246) and overstained sites (N = 9) were analyzed together as stained mucosa. Sensitivity, specificity, positive predictive value, and negative predictive value statistics were estimated for both visible lesions (before staining) and unstained areas (after staining) for identifying high grade squamous dysplasia or carcinoma. Sensitivity (true-positives/[true-positives + false-negatives]) was the proportion of high grade dysplasia or carcinoma biopsies that came from visually abnormal sites; specificity (true-negatives/[true-negatives + false-positives]) was the proportion of other histologies that came from visually normal sites; positive predictive value (true-positives/[true-positives + false-positives]) was the proportion of visually abnormal sites that contained high grade dysplasia or carcinoma; and negative predictive value (true-negatives/[true-negatives + false-negatives]) was the proportion of visually normal sites that did not contain high grade dysplasia or carcinoma. Ninety-five percent confidence intervals (95% CI) (the range of values expected 95% of the time) were calculated around these estimates. The difference between the sensitivity estimates before and after staining was tested by calculating the Z value.[33]

In the analysis by patient, the proportion of patients with high grade squamous dysplasia or carcinoma who were identified only after staining and the proportion of patients who had additional, larger, or more clearly defined high grade dysplastic or cancerous lesions after staining were calculated.

RESULTS

Two hundred and twenty-five adults participated in the endoscopic examinations, including 115 men (51%) and 110 women (49%), with an average age of 53 years (range, 40–70 years). Eight of the 213 participants who completed the baseline interview (3.8%) admitted difficulty swallowing or pain on swallowing. None reported a previous allergy to iodine.

All 225 patients completed endoscopy successfully. None of the patients had endoscopic evidence of gastric heterotopia or Barrett's metaplasia. There were no complications during or after the examinations. Two hundred and twenty-three patients had at least 1 satisfactory esophageal biopsy. Categorized by their worst esophageal biopsy diagnosis, 87 patients had normal squamous mucosa, 22 had esophagitis, 29 had mild dysplasia, 31 had moderate dysplasia, 35 had severe dysplasia, and 19 had invasive squamous cell carcinoma. None of the esophageal biopsies showed histologic evidence of Barrett's metaplasia. Forty-five patients had biopsies taken from the stomach. Categorized by their worst gastric biopsy diagnosis, 13 patients had normal glandular mucosa, 15 had gastritis, 1 had low grade dysplasia, 3 had high grade dysplasia, and 13 had invasive adenocarcinoma. All biopsies showing glandular dysplasia or adenocarcinoma came from the gastric cardia. In all, 125 of the 223 patients (56%) had biopsies showing esophageal or gastric dysplasia or carcinoma. Three of the eight symptomatic patients had invasive esophageal squamous cell carcinoma, two had invasive gastric adenocarcinoma and esophagitis, one had esophagitis alone, and two had only normal biopsies.

In 77 consecutive patients, we recorded more detailed information regarding several aspects of the staining procedure.[34] In these patients, the average duration of endoscopy was 13 minutes (range, 5–31 minutes). Iodine spraying took 1–2 minutes, and maximum staining was observed 0.5–1.0 minute after spraying was complete. Staining intensity and duration varied considerably among patients, but on average staining was 75% of maximum at 2 minutes, 50% at 3 minutes, 25% at 5 minutes, and 0% at 8 minutes after spraying. Only four patients needed restaining to complete adequate photographs and biopsies. On average, the iodine staining procedure (including spraying, observation, and photography) added 5–6 minutes to each endoscopy.

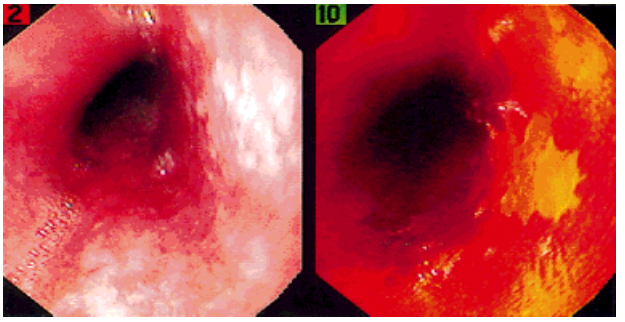

FIGURE 1. Before staining, a focal area of irregular mucosa was observed at 32 cm (3:00 position). After staining, the lesion was of similar size, but had more distinct borders. Biopsies showed moderate dysplasia.

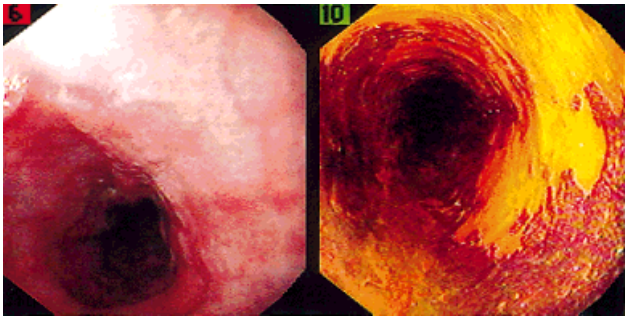

FIGURE 2. Before staining, a plaque was identified at 23–24 cm (11:00–4:00 position). After staining, the lesion was observed to extended from 23–28 cm (10:00–6:00 position). Multiple biopsies showed severe dysplasia.

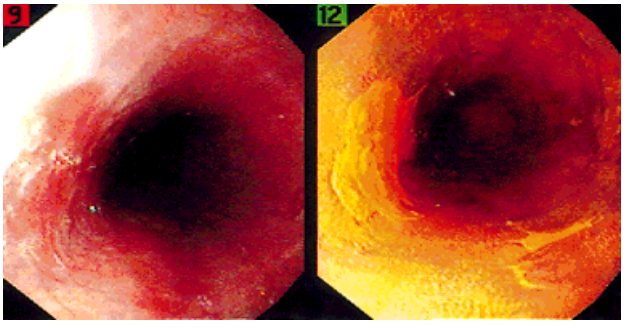

FIGURE 3. Before staining, a large broad-based erosion was observed at 28 cm (8:00–10:00 position). After staining, the same lesion was outlined more clearly and a satellite lesion (5:00 position) became apparent. Biopsies of both lesions showed severe dysplasia.

It was immediately apparent that staining gave additional information on the presence and extent of mucosal abnormalities. In some cases, a visible abnormality that was observed before staining became an unstained lesion (USL) that had a similar size but clearer borders (Fig. 1). In other cases, visible lesions observed before staining became larger USLs (Figs. 2–5), occasionally with separate satellite foci (Fig. 3) or a "mosaic" pattern of multiple large unstained areas

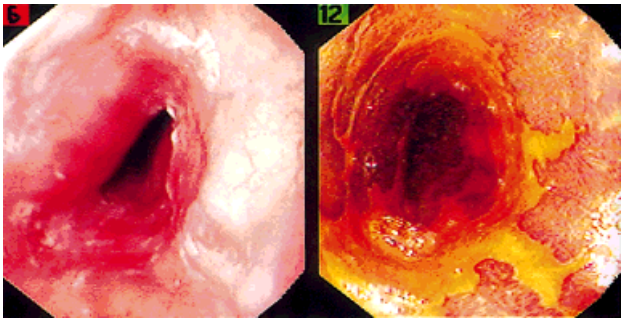

FIGURE 4. Before staining, a large broad-based erosion was observed at 25–29 cm (2:00–7:00 position). After staining, the lesion was longer and circumferential, with thin bridges connecting large unstained areas. Multiple biopsies showed moderate dysplasia.

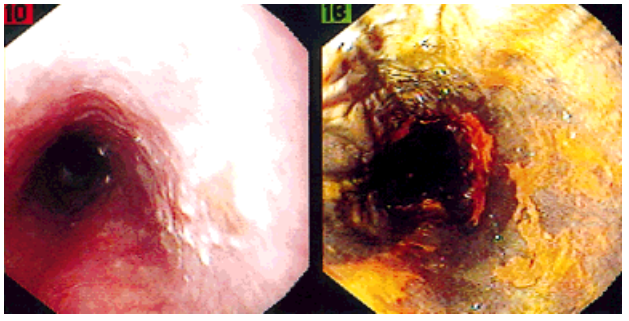

FIGURE 5. Before staining, a single punched-out erosion was noted at 28 cm (3:00 position). After staining, there were many unstained areas from 24–29 cm. Biopsies of several of the lesions showed severe dysplasia.

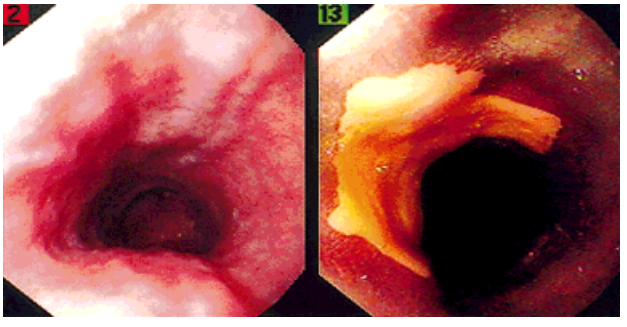

FIGURE 6. Before staining, the mucosa appeared normal. After staining, a prominent unstained lesion was observed at 20–22 cm. Multiple biopsies showed severe dysplasia.

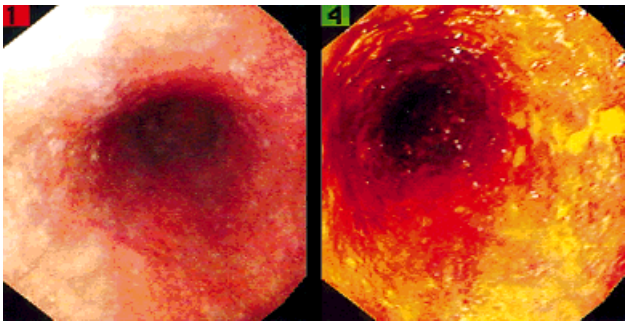

FIGURE 7. Before staining, the mucosa appeared normal. After staining, several small unstained areas (3:00 position) were noted. Biopsies showed normal mucosa.

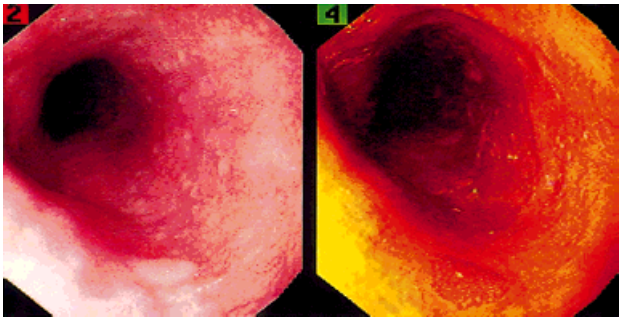

FIGURE 8. Before staining, a small white patch was identified at 30 cm (6:00 position). After staining, the same lesion stained more deeply than the surrounding mucosa. A biopsy showed glycogenic acanthosis.

(Figs. 4 and 5). In still other cases, staining of normal-appearing mucosa showed a single large USL (Fig. 6) or one or more small unstained areas that appeared to be clinically insignificant (Fig. 7). A few small white patches became overstained (Fig. 8), and were histologically consistent with glycogenic acanthosis. Of the 279 lesions observed before or after staining in these patients, 125 (45%) were identified only after staining, 71 (25%) appeared larger after staining, 74 (27%) appeared to be the same size, 1 (0.4%) appeared smaller, and 8 (3%) disappeared after staining. Of the 146 lesions observed before and after staining, 93 (64%) had clearer borders, 47 (32%) had borders that were equally clear, and 6 (4%) had less clear borders after staining.

Table 1 shows the correlation of endoscopic appearance before staining and biopsy diagnosis in the 508 squamous biopsy sites. All 20 biopsies containing carcinoma came from visible lesions, as did 65% of the biopsies containing severe dysplasia, 42% of the biopsies containing moderate dysplasia, and 32% of the biopsies containing mild dysplasia. The sensitivity of visible lesions for detecting areas of high grade (moderate or severe) squamous dysplasia or carcinoma was 62% (95% CI, 53%–71%), the specificity was 79% (95% CI, 75%–83%), the positive predictive value was 46% (95% CI, 39%–54%), and the negative predictive value was 88% (95% CI, 85%–91%).

Table 2 shows the same biopsy results stratified by mucosal staining pattern. All 20 biopsies containing carcinoma came from unstained areas, as did 96% of the biopsies containing severe dysplasia, 93% of the biopsies containing moderate dysplasia, and 63% of

the biopsies containing mild dysplasia. Three of the five biopsies of moderate or severe dysplasia that came from stained mucosa were control biopsies (near unstained lesions), and two were standard biopsies (from midesophageal sites away from unstained areas). The sensitivity of unstained areas for detecting high grade squamous dysplasia or carcinoma was 96% (95% CI, 92%–99%), the specificity was 63% (95% CI, 59%–68%), the positive predictive value was 43% (95% CI, 37%–49%), and the negative predictive value was 98% (95% CI, 96%–100%). One hundred of the 114 high grade dysplasias and carcinomas (88%) appeared larger or more clearly defined after staining.

The difference in the 62% sensitivity of visible lesions observed before staining and the 96% sensitivity of unstained areas observed after staining for detecting high grade squamous dysplasia or carcinoma was statistically significant ($P < 0.001$).

There was a positive relation between the size of unstained lesions and the presence of high grade squamous dysplasia or carcinoma. High grade dysplasia or carcinoma was found in 46 of 149 (31%) of the USLs $<$ 1 cm long, in 35 of 72 (49%) of those 1–5 cm long, and in 28 of 32 (88%) of those $>$ 5 cm in length. High grade dysplasia or carcinoma was present in 36 of 162 (22%) of the USLs involving $\leq$ 25% of the esophageal circumference and in 72 of 90 (80%) of the USLs involving $>$ 25% of the circumference.

Table 3 shows the impact of mucosal iodine staining on the identification of patients with high grade squamous dysplasia or carcinoma. In all 19 patients with carcinoma, the diagnostic lesion was identified before staining, but in 8 of the 35 patients with severe dysplasia (23%) and 17 of the 31 patients with moderate dysplasia (55%), the diagnostic lesion was observed and biopsied only after staining, and would have been missed if staining had not been used. In addition, 11 of the 58 patients whose worst histologic lesions were observed before staining had other separate foci of high grade dysplasia or carcinoma that were identified only after staining. Thus 36 of the 85 patients with high grade dysplasia or carcinoma (42%) had at least 1 of these lesions detected only after staining. Furthermore, 69 of these 85 patients (81%) had a high grade dysplastic or cancerous lesion that was observed to be larger after staining, and 78 (92%) had such a lesion whose margins were defined more clearly after staining. In all, 78 of the 225 patients who underwent endoscopy (35%) had additional, larger, or more clearly defined high grade dysplastic or cancerous lesions revealed by the staining procedure.

Staining did not improve visualization of gastric lesions.

DISCUSSION

In previous studies in the high risk population of Linxian, China, we found that high grade (moderate and severe) squamous dysplasia was the clinically important near-term precursor lesion of squamous esophageal carcinoma (it was the only histologic lesion associated with a significantly increased risk of developing invasive carcinoma in the first 3.5 years after biopsy)[35] and that most foci of high grade squamous dysplasia and invasive squamous carcinoma were associated with endoscopically visible lesions that could be targeted for biopsy.[6] However, in this latter study an important minority (27%) of the high grade squamous dysplasias were not identified endoscopically, and would have been missed if only the visible lesions had been biopsied. The current study was performed to determine whether we could improve our detection of the clinically important lesions (the high grade dysplasias and early invasive carcinomas) by mucosal iodine staining.

The basis of the iodine staining technique is that iodine reversibly stains glycogen brown.[22,36,37] In normal squamous esophageal mucosa, the superficial epithelium contains abundant glycogen, so the mucosa stains dark brown, but in abnormal mucosa, including areas of esophagitis, atrophy, keratinization, squamous dysplasia, and squamous carcinoma, the superficial epithelium often loses much of its glycogen, and remains partially or totally unstained.[8,17,18,22,23,26,38–41] Glandular mucosa, including normal gastric mucosa, gastric heterotopia, and Barrett's metaplasia, also appears unstained.[8,18,23,26,36,38,42] Foci of glycogenic acanthosis appear overstained,[18,22,38,43] and necrotic material in the base of an ulcer also can appear dark brown, occasionally with an unstained rim.[22,38]

The first use of iodine staining to detect mucosal abnormalities was by Schiller, who used this technique to highlight squamous lesions in the cervix.[44] Similar staining first was used in the esophagus by Voegeli,[7] Brodmerkel,[38] Northmann et al.,[36] and Toriie et al.[8] In recent years, esophageal mucosal iodine staining has become common in Japan and Europe, and several studies have reported that it improves detection and border discrimination of squamous neoplastic lesions.[9–26] However, the sensitivity and specificity of the technique for identifying these lesions have not been well defined,[45] and it has not been used widely in other countries, including China and the U. S.

The formula of the iodine stain that we used (12 g iodine + 24 g potassium iodide dissolved in 1000 mL of water) was taken from Endo and Ide.[18] This formula is slightly stronger than original Lugol's solution (1 g iodine + 2 g potassium iodide in water to 100 g; 1%

TABLE 1
Squamous Biopsy Results by Endoscopic Appearance Before Staining

Endoscopic appearance	Biopsy diagnosis (column percent)						
	Normal	Esophagitis	Mild dys	Mod dys	Sev dys	Carcinoma	Total
Visible lesion[a]	48 (17)	14 (34)	20 (32)	19 (42)	32 (65)	20 (100)	153 (30)
No visible lesion[b]	242 (83)	27 (66)	43 (68)	26 (58)	17 (35)	0 (0)	355 (70)
Total	290 (100)	41 (100)	63 (100)	45 (100)	49 (100)	20 (100)	508 (100)

Mild dys: mild dysplasia; Mod dys: moderate dysplasia; Sev dys: severe dysplasia.
[a] Includes all endoscopic categories except visually normal mucosa.
[b] Visually normal mucosa.

TABLE 2
Squamous Biopsy Results by Mucosal Staining Pattern

Mucosal staining pattern	Biopsy diagnosis (column percent)						
	Normal	Esophagitis	Mild dys	Mod dys	Sev dys	Carcinoma	Total
Unstained	80 (28)	24 (59)	40 (63)	42 (93)	47 (96)	20 (100)	253 (50)
Stained	210 (72)[a]	17 (41)	23 (37)	3 (7)	2 (4)	0 (0)	255 (50)
Total	290 (100)	41 (100)	63 (100)	45 (100)	49 (100)	20 (100)	508 (100)

Mild dys: mild dysplasia; Mod dys: moderate dysplasia; Sev dys: severe dysplasia.
[a] Includes all nine biopsies from overstained areas.

TABLE 3
Impact of Mucosal Staining on the Identification of Patients with High Grade Squamous Dysplasia or Carcinoma

Worst patient diagnosis	Diagnostic lesion observed before stain	Diagnostic lesion observed only after stain	Diagnostic lesion not observed before or after stain	Total
Mod. dys	13 (42)[a]	17 (55)	1 (3)	31 (100)
Sev. dys	26 (74)	8 (23)	1 (3)	35 (100)
Carcinoma	19 (100)	0 (0)	0 (0)	19 (100)
Total	58 (68)	25 (29)	2 (2)	85 (100)

Mod dys: moderate dysplasia; Sev dys: severe dysplasia.
[a] Row percentage.

weight/weight iodine),[46] and has been called 1.2% Lugol's by some authors, referring to the elemental iodine content, and 3% Lugol's by other authors, referring to the total (iodine + potassium iodide) iodine content. Until standard nomenclature is adopted, authors should be encouraged to specify the formulas of the stains that they use.

In our patients, mucus rarely was a problem, so we did not find it necessary to pretreat the mucosa with a water rinse or a mucolytic agent.[15,18] The majority of the USLs were clearly visible for 5–8 minutes, giving ample time for photographs and biopsies or for most focal therapy procedures.[34] If the stain did begin to fade, restaining was easily accomplished and effective. In a few patients, the entire esophagus did not stain well, for unknown reasons. Only occasional patients reported any discomfort after the procedure. This mild discomfort appeared to be caused by gastric distention and/or reflux of iodine, and was minimized by careful suctioning of air and iodine from the stomach before removal of the endoscope. We did not find it necessary to spray the mucosa with sodium thiosulfate solution after the procedure to reduce pain.[25,47]

One of the great advantages of iodine staining was how easy it was to interpret. The visible lesions we identified before staining often were quite subtle, and some certainly would have been missed if we had not had expert endoscopists looking specifically for such lesions. However, after staining the USLs were nearly always quite obvious, contrasting sharply with the surrounding stained mucosa, so they were much easier to identify and target for biopsy. This ease of identification should be especially helpful for inexperienced endoscopists or those who encounter early neoplastic lesions only occasionally.

We did not biopsy very small USLs that we considered clinically insignificant. This was not because we believe there is a lower limit to the size of dysplastic or cancerous foci,[25,48] but rather because, in our

high risk population, we had so many larger USLs to biopsy and previous authors have found that the likelihood of USLs being neoplastic increases with size.[48,49] Our data also show a positive correlation between USL size and the probability of clinically significant neoplasia. However, in a lower risk population, with fewer USLs, the smaller unstained foci also may be important to sample.

In our study, mucosal iodine staining dramatically improved our ability to detect the presence and extent of most squamous precursor lesions and invasive squamous carcinomas. In many cases, staining showed lesions to be larger than previously appreciated (Figs. 2–5) or it made the borders of visible lesions more clear (Figs. 1–5). In other cases, staining revealed prominent lesions that were not previously observed (Fig. 6). The clinical value of staining varied depending on which components of visibility (lesion detection, lesion extent, or clarity of lesion borders) and which grades of neoplastic disease (mild dysplasia, moderate and severe dysplasia, or invasive carcinoma) were evaluated. Staining did not improve our detection of invasive squamous carcinoma, because all our carcinomas were observed as mucosal abnormalities before staining, but it greatly improved our detection of all grades of squamous dysplasia and it improved our visualization of lesion extent and border clarity in both invasive and noninvasive neoplastic disease. Overall, the addition of iodine staining increased the detection rate of the clinically important lesions (the high grade dysplasias and carcinomas) from 62% to 96% (significance of difference, $P < 0.001$), and it improved our ability to see the true size and borders of nearly 90% of these abnormalities.

Analyzed by patient, nearly 25% of the patients with severe dysplasia and over 50% of those with moderate dysplasia would have been underdiagnosed if staining had not been used. Staining revealed additional, larger, or more clearly defined high grade dysplastic or cancerous lesions in 35% of the 225 endoscoped patients. This is the proportion of patients who might be treated differently (and might potentially benefit) because of the use of the stain. This proportion would be expected to be lower in a population with a lower prevalence of neoplastic disease.

The most striking finding in our study was the very high sensitivity of USLs for detecting high grade squamous dysplasia and invasive squamous carcinoma. This sensitivity may have been somewhat overestimated because the unstained lesions were biopsied more thoroughly than the stained mucosa (so there was a greater chance of finding the target histologies, if present, in the former than in the latter). Also, we did not study patients who had negative balloon cytology examinations, and such patients might (or might not) have smaller dysplastic or invasive lesions that might be more difficult to observe. Conversely, our sensitivity may have been somewhat underestimated because we did not biopsy small "insignificant" USLs, and other authors have shown that such lesions occasionally can contain dysplasia or carcinoma.[11,18,21,48,49] We believe that our study, as performed, clearly shows that mucosal iodine staining is a very sensitive technique for detecting the precursor and early invasive lesions of squamous esophageal carcinoma.

In our patients, USLs were not very specific for squamous dysplasia or carcinoma. This was not surprising, because lack of iodine staining merely means that the superficial epithelium does not contain abundant glycogen, and there are many reasons other than the presence of dysplasia or carcinoma for this to occur, including inflammation, atrophy, surface keratinization, or the presence of glandular mucosa.[8,17,18,22,23,26,36,38–42] However, this lack of specificity is not particularly worrisome because endoscopic identification of USLs is only half of the diagnostic method. The other half is taking targeted biopsies of the unstained areas, and these biopsies usually are quite reliable for distinguishing between high grade neoplasia and the other histologies associated with USLs. Thus, endoscopic inspection after iodine staining and endoscopic biopsy of the USLs are complementary procedures that together form a highly sensitive and specific method for detecting clinically important esophageal squamous neoplasia.

Our findings agree well with those of previous studies, especially if we make allowance for differences in diagnostic nomenclature and the problem of missing data. One difficulty with comparing the results of previous studies is the variable use of the terms dysplasia and carcinoma to describe noninvasive squamous neoplasia. Some authors (including ourselves) call all intraepithelial neoplastic lesions "dysplasia" and reserve the term "carcinoma" for invasive lesions, others use both "dysplasia" and "intraepithelial carcinoma" for noninvasive neoplastic lesions,[17,18,21,22] and still others do not use the term "dysplasia" at all and consider all neoplastic cells in the epithelium to be "intraepithelial carcinoma."[50] Thus, comparing the findings of various authors concerning the visibility of "carcinoma" can be misleading, and we must try to compare reports after stratifying the data by invasion status. The second difficulty is the problem of missing data. Many studies of mucosal iodine staining have reported only the number of carcinomas detected before and after using the stain, and have not reported other biopsy results from USLs or biopsy findings from stained mucosa. This makes it impossible to calculate sensitivity, specificity,

TABLE 4
Endoscopic Detection of High Grade Intraepithelial and Invasive Esophageal Squamous Neoplastic Lesions before and after Mucosal Iodine Staining

	High grade intraepithelial lesions[a]				Invasive lesions				All lesions			
Study and year	No. of lesions	No. observed before stain	No. observed after stain	% observed only after stain[b]	No. of lesions	No. observed before stain	No. observed after stain	% observed only after stain	No. of lesions	No. observed before stain	No. observed after stain	% observed only after stain	Comment
Bogomoletz et al. (1989)[14]	4	2	4	50%	61	55	61	10%	65	57	65	12%	Esophagectomy specimens
Misumi et al. (1990)[15]	10	8	10	20%	7	6	7	14%	17	14	17	18%	
Nabeya et al. (1990)[16]	10	8	10	20%	22	22	22	0%	32	30	32	6%	
Shiozaki et al. (1990)[17]	4	1	4	75%	6	3	6	50%	13	4	13	69%	Screening H & N CA patients
Chisolm et al. (1992)[19]	3	3	3	0%	3	2	3	33%	6	5	6	17%	Screening H & N CA patients
Sugimachi et al. (1992)[21]	3	3	3	0%	29	29	29	0%	32	32	32	0%	
Mori et al. (1993)[22]	7	1	7	86%	25	25	25	0%	32	26	32	19%	Esophagectomy specimens
Ina et al. (1994)[23]									8	3	8	63%	Screening H & N CA patients
Yokoyama et al. (1995)[25]									36	12	36	67%	Screening asxic alcoholics
Fagundes et al. (1997)[51]	2	0	2	100%	2	2	2	0%	4	2	4	50%	Screening asxic alcoholics
Meyer et al. (1997)[26]	5	3	5	40%	12	11	12	8%	17	14	17	18%	Screening sxic alcoholics
Total of previous studies	48	29	48	40%	167	153	167	8%	262	197	262	25%	
Current study	94	50	89	41%	20	20	20	0%	114	70	109	34%	Screening asxic individuals
Total	142	79	137	41%	187	173	187	7%	376	267	371	28%	

H & N A CA: head and neck carcinoma; asxic: asymptomatic, sxic: symptomatic.

[a] Includes moderate dysplasia, severe dysplasia, carcinoma in situ, and intraepithelial carcinoma.

[b] Percentage observed only after stain = (no. observed after stain - no. observed before stain)/total no. of lesions

or predictive value statistics for these series. Future authors should be encouraged to report all their biopsy results, from both stained and unstained mucosa, and to distinguish clearly between invasive and noninvasive lesions.

The results of previous studies reporting cases identified before and after mucosal iodine staining are shown in Table 4, stratified when possible by invasion status. In aggregate, the findings of these studies are similar to our own, that iodine staining usually is not required for detecting invasive squamous carcinoma, but it is essential for detecting many clinically important intraepithelial lesions. Thus, the perceived value of staining in different studies will depend a great deal on whether invasive or noninvasive lesions are more prevalent in the patients being evaluated. If the majority of the patients in a study are symptomatic (as in the series of Meyer et al.[26]), the majority of the lesions will be invasive carcinoma and the value of staining for identifying these lesions will appear to be relatively small. But if the majority of the patients are asymptomatic (as in the series of Yokoyama et al.[25] and in the current study), the majority of the lesions will be noninvasive and the value of staining will appear to be much more significant. We believe it is clear, from the previous studies and our own, that mucosal iodine staining is absolutely essential for endoscopically screening or confirming suspected lesions in asymptomatic individuals.

Table 5 shows the sensitivity, specificity, and predictive value statistics of the previous studies of mucosal iodine staining that have reported the data needed to calculate these statistics. Although the exact data reported varies from study to study (carcinoma only vs. high grade dysplasia and carcinoma; worst histologic diagnosis per patient vs. worst histologic diagnosis per biopsy site), all the studies have found that USLs have a very high (91–100%) sensitivity for detecting clinically important squamous neoplasia, similar to the current results. Also consistent with these high sensitivity figures are the results of previous esophagectomy studies that show a close correlation between the location and size of USLs and the location and size of histologic dysplasia and carcinoma.[15]

Of course detection is only one aspect of lesion visibility. Accurate visualization of lesion extent, including the presence of contiguous spread and satellite le-

TABLE 5
Sensitivity, Specificity, and Predictive Value Statistics in Studies Evaluating Mucosal Iodine Staining

Study and year	Definition of a positive observation	Total no. of observations	No. of positive observations	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Comment
Toriie et al. (1975)[8]	Bx dx = CA	148 bx sites	11	91%	72%	21%	99%	
Shiozaki et al. (1990)[17]	Patient dx = CA	179 patients	9	100%	64%	13%	100%	Screening H&N CA patients
Mori et al. (1993)[22]	Lesion dx = HGD − CA	115 lesions	47	96%	40%	52%	93%	Esophagectomy specimens
Ina et al. (1994)[23]	Patient dx = CA	127 patients	8	100%	57%	14%	100%	Screening H&N CA patients
Fagundes et al. (1997)[51]	Bx dx = HGD − CA	401 bx sites	4	100%	95%	16%	100%	Screening asxic alcoholics
Meyer et al. (1997)[26]	Bx dx = HGD − CA	125 bx sites	17	100%		14%		Screening sxic alcoholics
Current study	Bx dx = HGD − CA	508 bx sites	114	96%	63%	43%	98%	Screening asxic individuals

Bx: biopsy; dx: diagnosis; CA: carcinoma; H&N CA: head and neck carcinoma; HGD: high grade (moderate or severe) dysplasia; asxic: asymptomatic; sxic: symptomatic.

sions, is of critical importance for deciding between focal therapy and esophagectomy and for planning the extent and evaluating the completeness of either of these treatments. The clarity of lesion margins also is important for targeting focal therapy. All authors who have commented on these aspects agree with our findings that visualization of lesion size and borders is nearly always improved by iodine staining, both in invasive and in noninvasive neoplastic disease.[11,15,16,18–22,24,26,49]

None of the patients in our study had endoscopic or histologic evidence of Barrett's metaplasia, so we cannot comment on the usefulness of iodine staining for detecting or delineating such lesions. Theoretically, iodine staining should highlight any border between normal squamous mucosa (which stains) and glandular mucosa (which does not stain). Previous studies have shown that iodine staining accentuates the normal squamocolumnar junction[36] and the presence of gastric heterotopias.[23,26] Some[42] but not all[52] authors have found it useful for visualizing Barrett's mucosa as well. There is no evidence that iodine staining can identify or highlight foci of glandular dysplasia or adenocarcinoma within Barrett's mucosa.

An effective early detection and treatment program for squamous esophageal carcinoma will require screening of asymptomatic high risk individuals. A successful screening program most likely will need a sensitive primary screening test that is acceptable to asymptomatic people, a secondary test that can confirm and localize precursor and early invasive lesions, accurate estimation of the depth of invasive lesions to determine which are amenable to focal therapy, and one or more curative treatments that are acceptable to asymptomatic patients. Because of its high sensitivity for the target lesions of screening (high grade squamous dysplasia and curable early invasive squamous carcinoma), we believe that endoscopy with mucosal iodine staining has potential applications in all of these areas. In some very high risk situations, such as screening patients with previous head and neck carcinomas, endoscopy with iodine staining may itself be practical as a primary screening test,[17,23,53] and in other settings it can be used as a gold standard against which other less costly, less invasive screening techniques can be evaluated.[27] In nearly all situations, endoscopy with iodine staining should be an excellent procedure for a secondary test to confirm and localize squamous abnormalities detected by other methods. For staging, staining can outline the lesions of interest for focal imaging systems such as endoscopic ultrasonography using a catheter probe.[54,55] And for focal therapy, staining is indispensable for its accurate delineation of lesion borders.[56–61] Finally, if esophagectomy is required, iodine staining can assist in defining the best proximal resection margin.[19,20,22]

In addition to its utility in clinical practice, mucosal iodine staining should be valuable in several research settings, including studies of the natural history of squamous dysplasia[62] and studies of chemoprevention or other interventions that use high grade squamous dysplasia as an intermediate endpoint for invasive squamous cell carcinoma.

In summary, we believe that mucosal iodine staining should be used whenever optimal visualization of esophageal squamous mucosal abnormalities is required. In our experience, this procedure is safe, inexpensive, simple and rapid to perform, easy to interpret, and highly sensitive for all clinically important squamous neoplasia. It is essential for detecting many squamous dysplasias and for determining the full extent of both invasive and noninvasive squamous neoplastic disease. We recommend mucosal iodine staining as a routine procedure in high risk populations and whenever an unexplained squamous mucosal abnormality is observed in lower risk individuals.

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